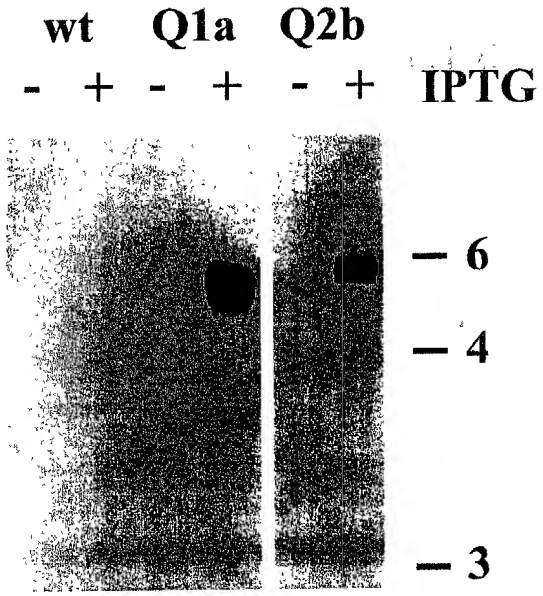
Figure 1:



HCV E2 proteins detected with mMAb E2G

Sequence of central fragment for HCV E2 vaccinia constructs Q1a, Q1b, Q2a, & Q2b compared to representative sequences of the appropriate HCV genotypes. Accession numbers for the representative sequences of each genotype are as follows HCV 1A = M62321, HCV 1B = D10750, HCV 2A = D00944, HCV 2B = D10988. Phlyogenetic analysis performed with CLUSTALV and DNAPARS program of the PHYLIP package.

Sequences amplified from central region of HCV E2 vaccinia virus clones

```
>hcv-1a3, (Q1a)
   CTCAACTGGATTCACCAAAGTGTGCGGAGCGCCCCCCTGTGTCATCGGAGGGGCGGG
CAACAACACCTT       GCGCTGCCCCACTGATTGTTTCCGCAAGCATCCGGAAGCCAC
GTACTCTCGGTGCGGCTCCGGTCCCTGGATTACGCCCAGGTGCCTGGTc

>hcv-1b8, (Q1b)
   TGGCACAGGGTTCACCAAGACGTGTGGGGCCCCCCCATGTAACATCGGGGGGGTCGG
CAATAACACCTT       GACTTGCCCCCACGGACTGTTTCCGGAAGCACCCCGAGGCCAC
TTACACCAAATGTGGTTCGGGGCCTTGGCTGACACCTAGGTGCATAGTt

>hcv-2a-25, (Q2a)
CTCCACTGT TTCACCAAAACTTGCGGCGCACCACCCTGCCGCATCAGAGCTGACTT
TAATGCCAGCACggaCCTGCTGTGCCCCACGGACTGTTTCAGGAAGCATCCTGAAGCCAC
TTACATCAAATGTGGCTCTGGGCCCctgtgacgccaaagtgcctgata

>HCV-2B-1,   (Q2b)
TGGGACTGGGTTCACTAAGACATGCGGTGCACCACCTTGCCGCATTAGGAGGGACTG
CAACGGAACCCTcgaCCTATTGTGCCCCACAGACTGTTTCAGAAAGCACCCAGATACTAC
CTACCTTAAGTGTGGAGCGGGGCCTTGGTTGACCCCCAAATGCATGGTa
```

Figure 2

```
Name                                        Sequences
----                                        ---------

HCV-1a        CTCAACTGGA TTCACCAAAG TGTGCGGAGC GCCTCCTTGT GTCATCGGAG GGGCGGGCAA
HCV-Q1a-FR    .......... .......... .......... ...C..C... .......... ..........
HCV-1b        TAGT.....G .....T..GA C........G C..C..G... AA......G. ...TC..T..
HCV-Q1b-FR    TGGC..A..G ........GA C...T..G.. C..C..A... AA......G. ...TC.....
HCV-2a        ...C.....C .A......GA CT.....C.. A..A..C..C CG...TA... CT.ACTT...
HCV-Q2a-FR    ...C....-T .........A CT.....C.. A..A..C..C CG....A... CT.ACTTT..
HCV-2b        .GGG.....G .....T..GA CA.....T.. A..A.....C CG...TA.GA AA.ACTA...
HCV-Q2b-FR    TGGG.....G .....T..GA CA.....T.. A..A.....C CG...TA.GA ...ACT....

HCV-1a        CAACACC--- ---CTGCACT GCCCCACTGA TTGCTTCCGC AAGCATCCGG ACGCCACATA
HCV-Q1a-FR    .......... ...T...G.. .......... ...T...... .......... .A.....G..
HCV-1b-       .CG....... ...T..AT.. .......G.. C........G .....C..C. .G..T..T..
HCV-Q1b-FR    T......... ...T..ACT. .......G.. C..T.....G .....C..C. .G.....T..
HCV-2a        TGC..G.ATG GACT..TTG. .......G.. C..T..TA.G ........T. .TA....C..
HCV-Q2a-FR    TGC..G.ACG GAC....TG. .......G.. C..T...A.G ........T. .A.....T..
HCV-2b        ..G...TATC GATT.ATTG. .......A.. C..T..TA.G .....C..A. .T..T..C..
HCV-Q2b-FR    .GGA...CTC GAC..ATTG. .......A.. C..T...A.A .....C..A. .TA.T..C..

HCV-1a        CTCTCGGTGC GGCTCCGGTC CCTGGATCAC ACCCAGGTGC CTGGTC
HCV-Q1a-FR    .......... .......... .......T.. G......... ......
HCV-1b        .A.AAAA..T .....G..G. .....T.G.. ...T...... ..A..A
HCV-Q1b-FR    .A.CAAA..T ..T..G..G. .T...C.G.. ...T...... A.A..T
HCV-2a        .ATCAAA..T .....T..G. .....C.... G..A...... ...A..
HCV-Q2a-FR    .ATCAAA..T .....T..G. ..CT.-.G.. G..A.A.... ...A.A
HCV-2b        TCT.AA...T ..AG.A..G. .T...T.A.. T......... .....A
HCV-Q2b-FR    .CT.AA...T ..AG.G..G. .T...T.G.. C....AA... A....A
```

One most parsimonious tree found:

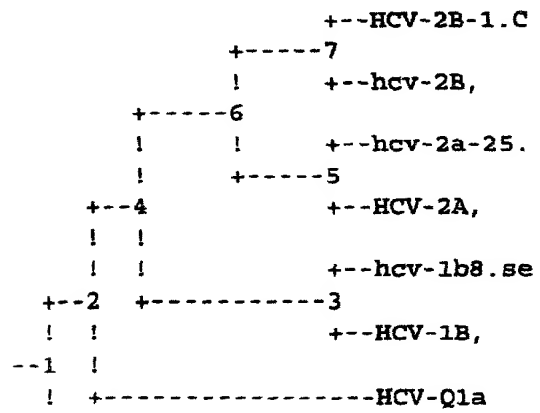


Figure 3

Figure 4

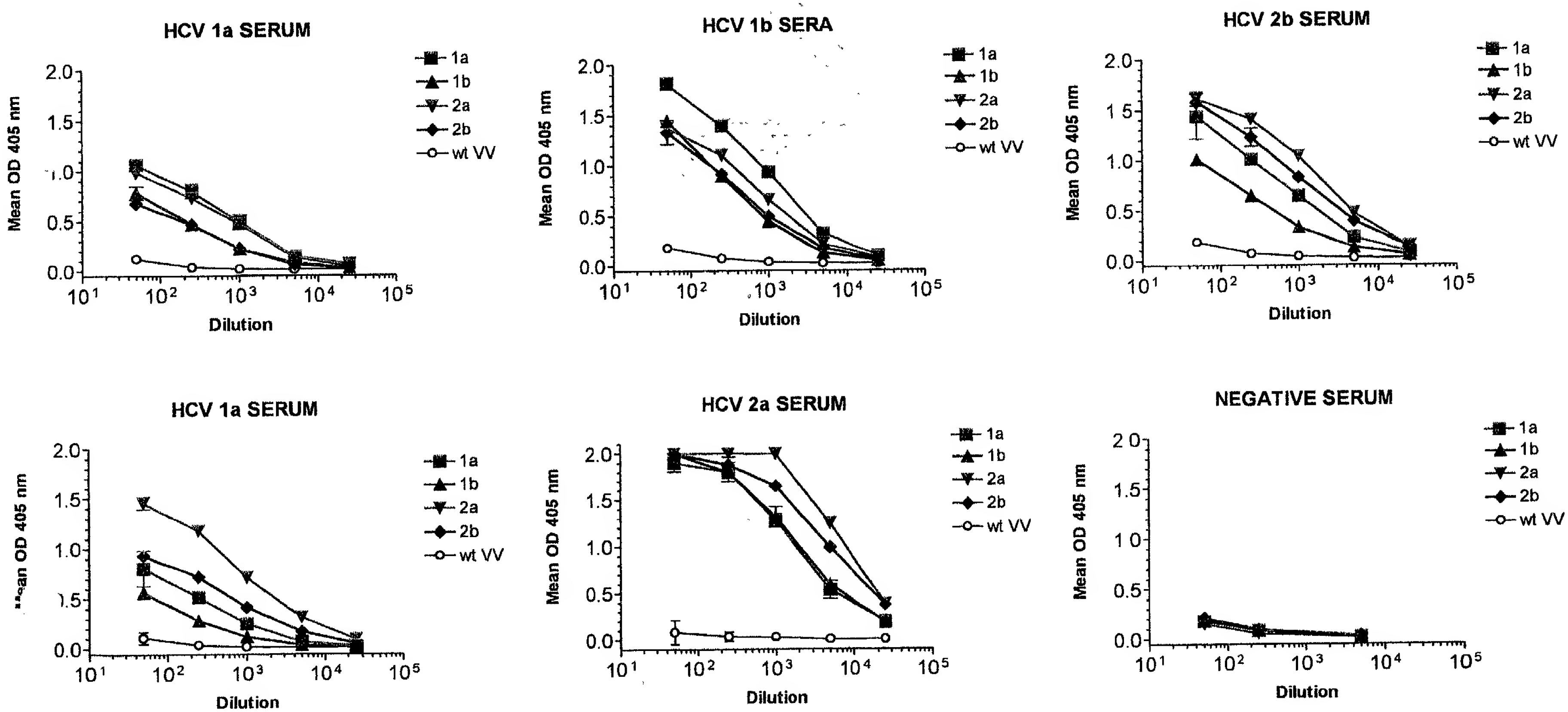

HCV 1a SERUM
HCV 1b SERA
HCV 2b SERUM
HCV 1a SERUM
HCV 2a SERUM
NEGATIVE SERUM
Mean OD 405 nm
Dilution
1a
1b
2a
2b
wt VV

Figure 5. Reactivity obtained with 12 HCV 2b Sera

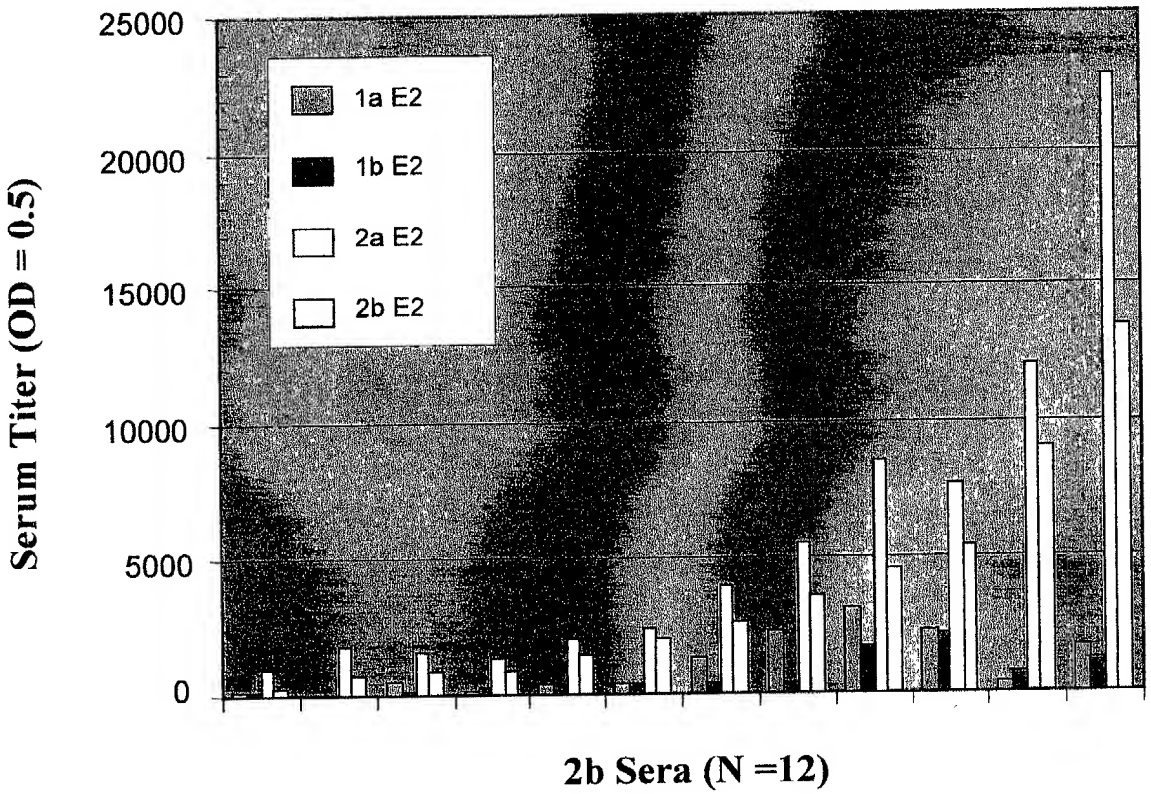
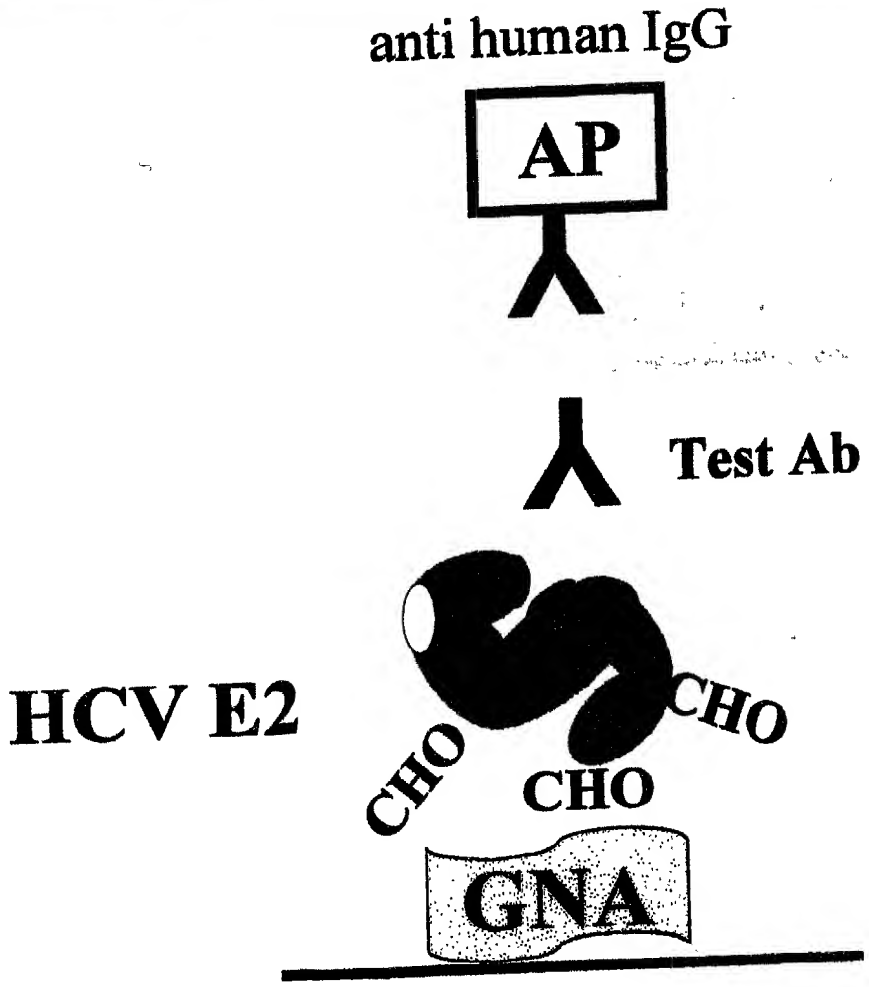


Figure 6

Binding Analysis of HCV HMAbs



- Coat plates with GNA lectin
- Add E2 containing protein extracts
- Add MAb
- Detect bound Ab with anti Human-AP

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K.G.H 1999

Figure 7

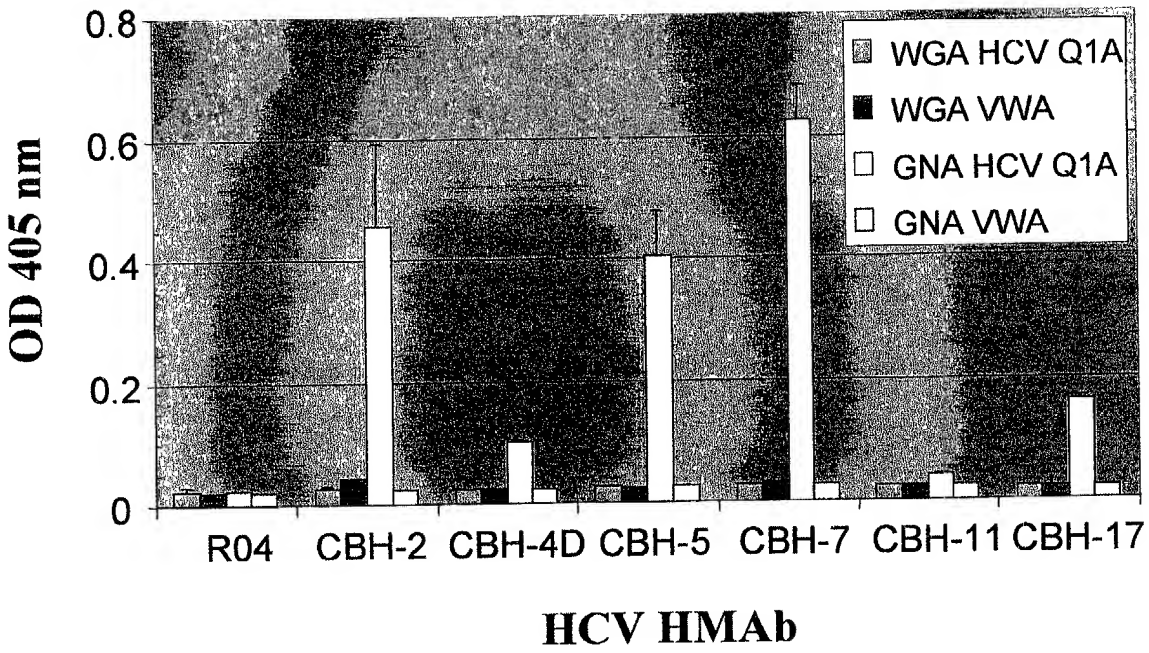

0.8
0.6
0.4
0.2
0
OD 405 nm
WGA HCV Q1A
WGA VWA
GNA HCV Q1A
GNA VWA
R04
CBH-2
CBH-4D
CBH-5
CBH-7
CBH-11
CBH-17
HCV HMAb

Figure 8

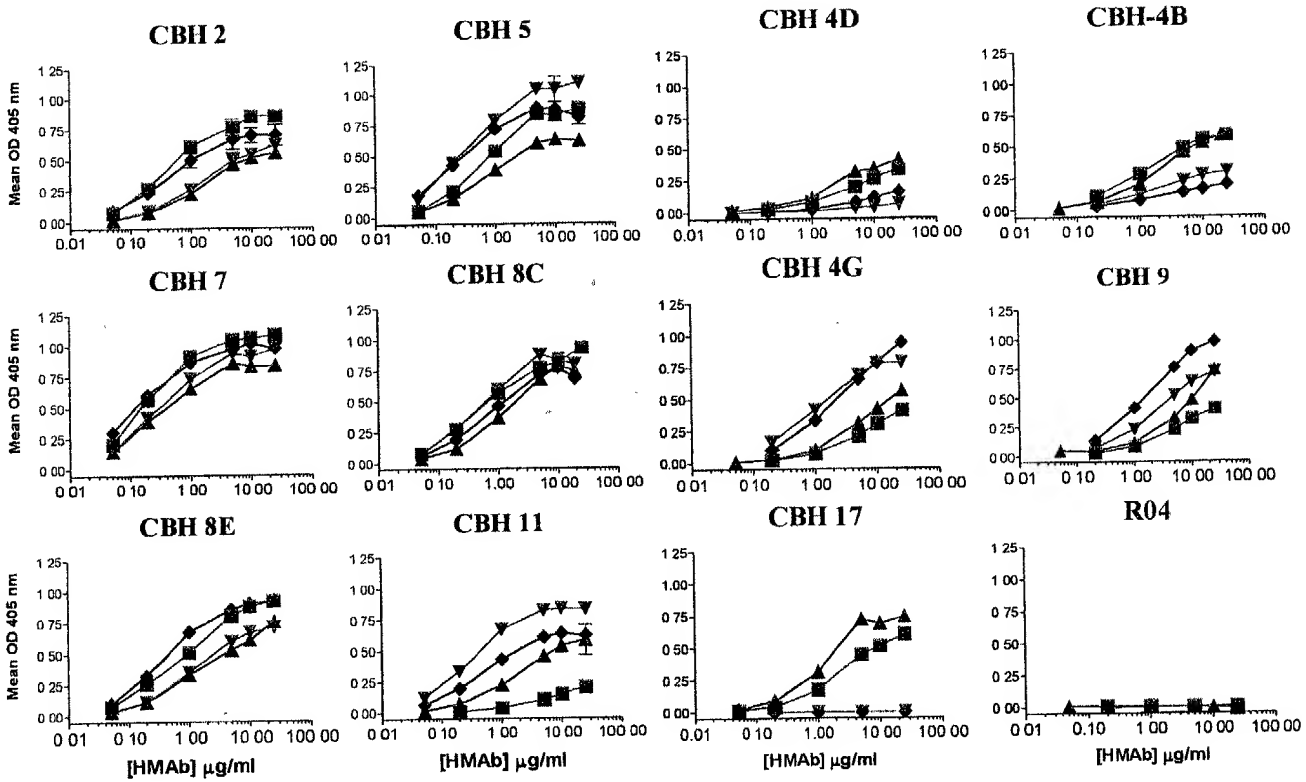
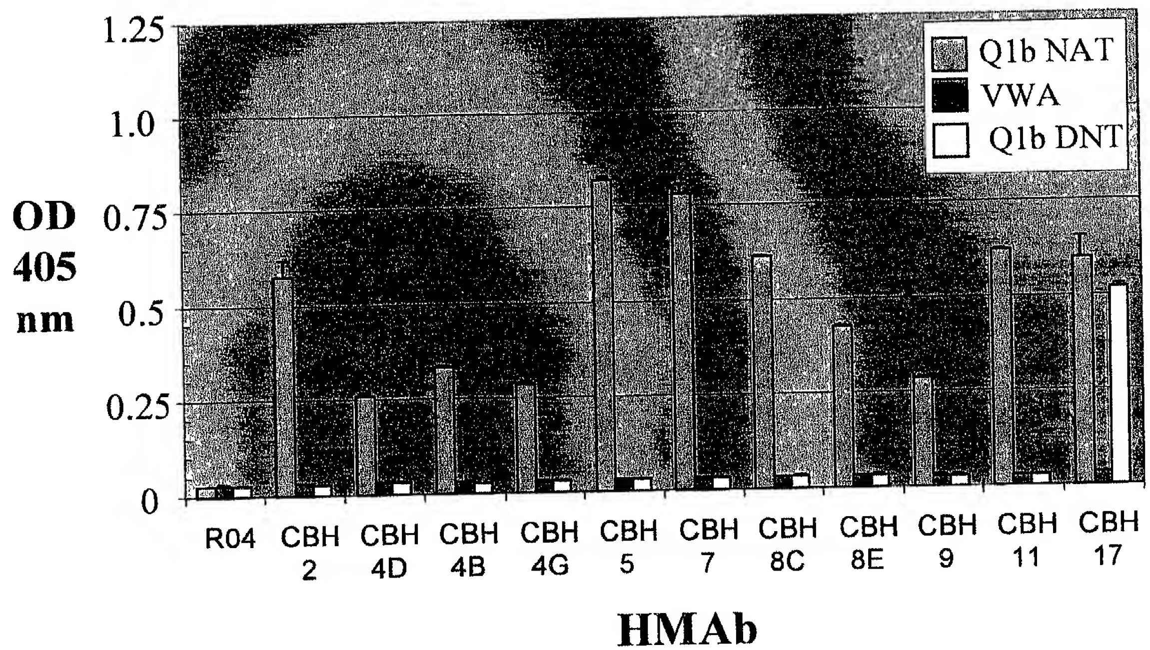


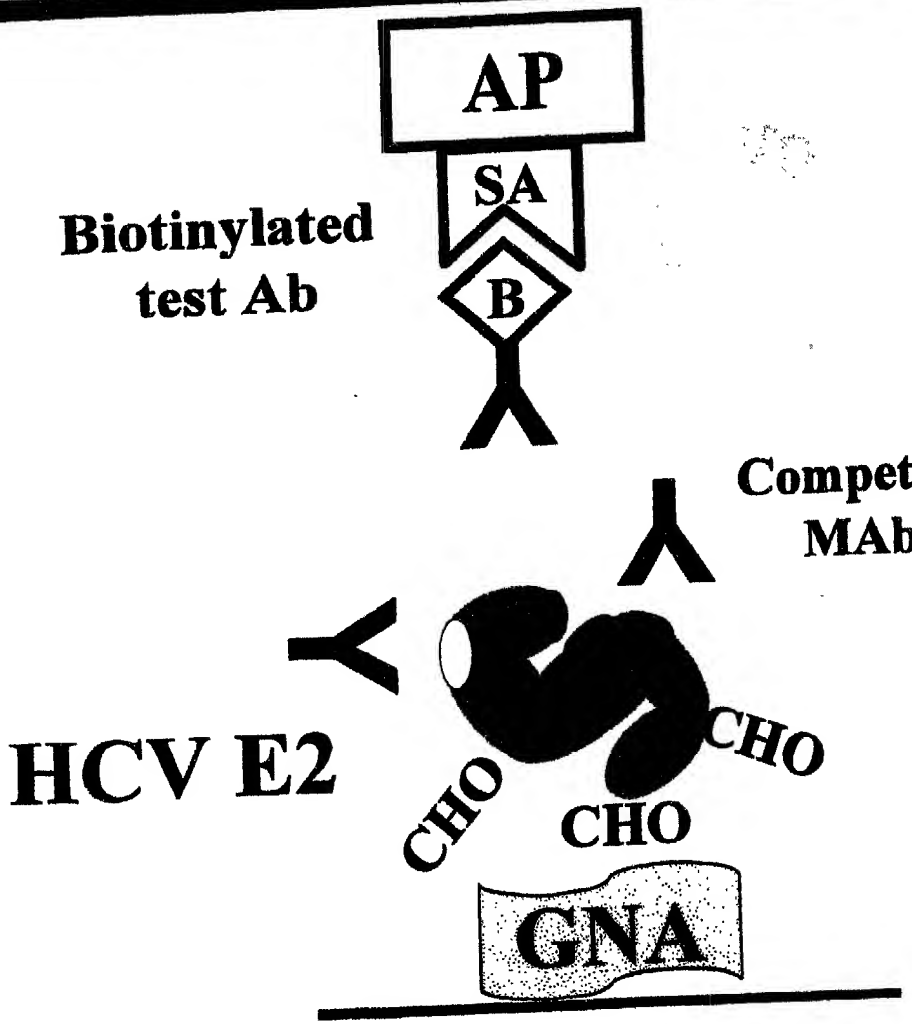
Figure 9



OD 405 nm
1.25
1.0
0.75
0.5
0.25
0
Q1b NAT
VWA
Q1b DNT
R04
CBH 2
CBH 4D
CBH 4B
CBH 4G
CBH 5
CBH 7
CBH 8C
CBH 8E
CBH 9
CBH 11
CBH 17
HMAb

Figure 10

Competition Analysis



- Coat plates with GNA lectin
- Add E2 containing protein extracts
- Add competing MAb
- Add biotinylated Test HMAb
- Detect bound Ab with Strep-AP

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K.G.H 1999

Figure 11

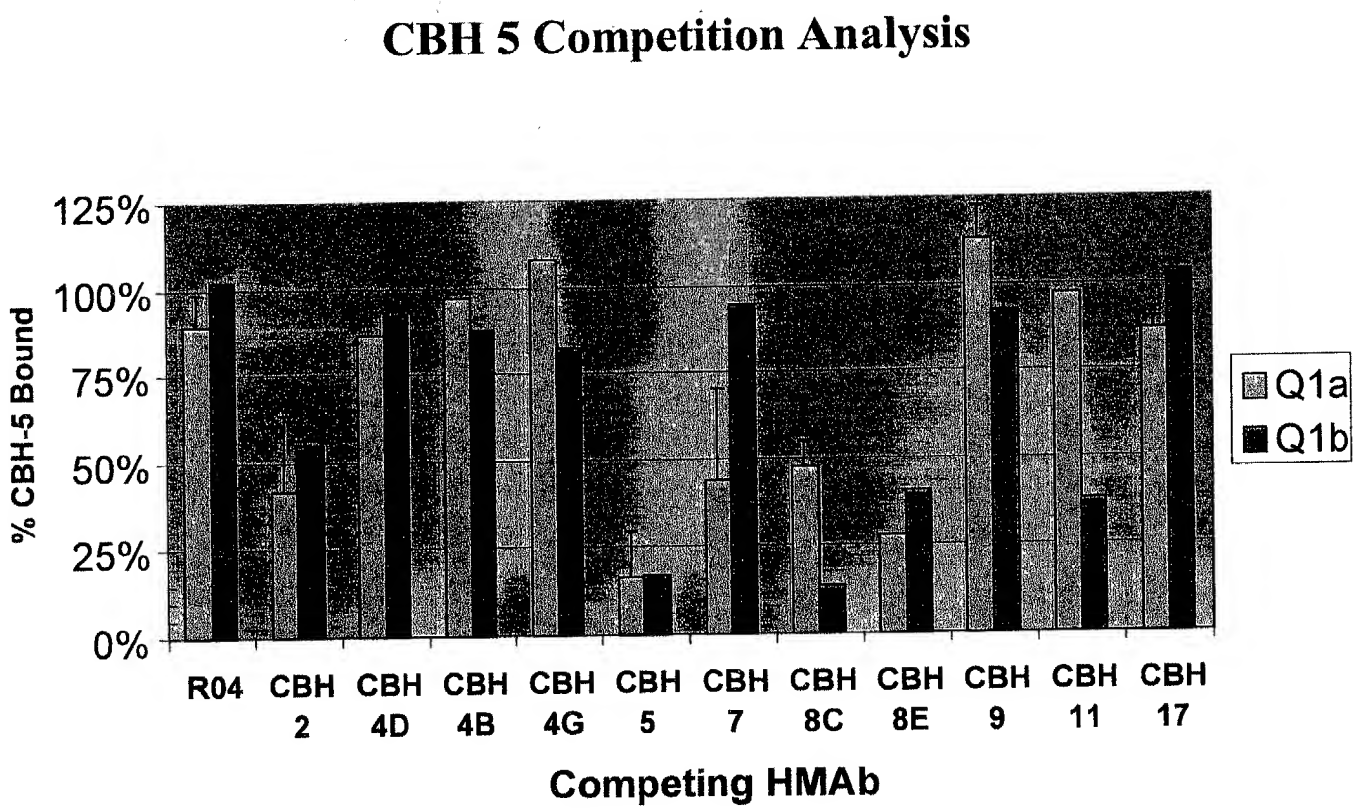


Figure 12

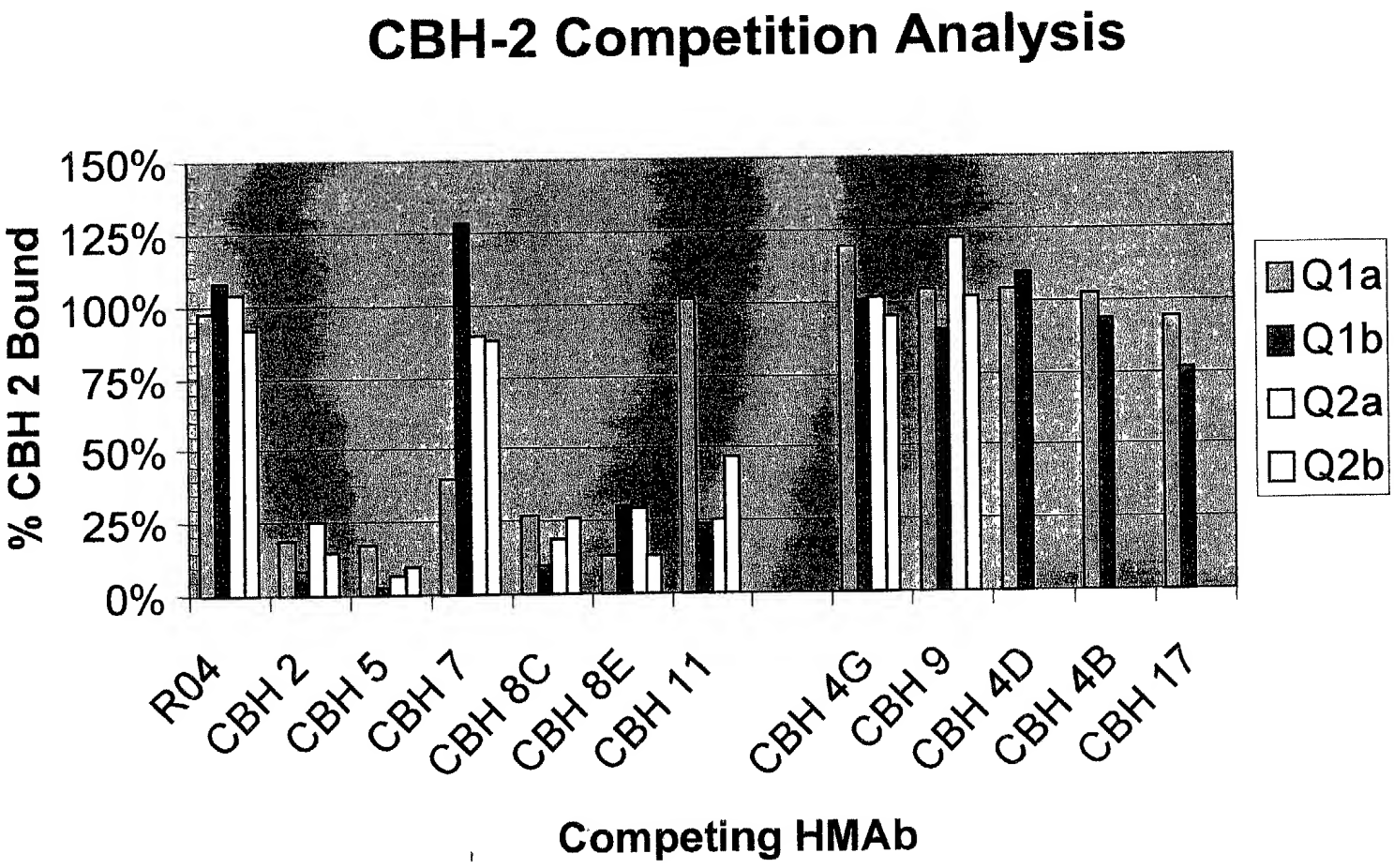

CBH-2 Competition Analysis
% CBH 2 Bound
150%
125%
100%
75%
50%
25%
0%
R04
CBH 2
CBH 5
CBH 7
CBH 8C
CBH 8E
CBH 11
CBH 4G
CBH 9
CBH 4D
CBH 4B
CBH 17
Competing HMAb
Q1a
Q1b
Q2a
Q2b

Figure 13

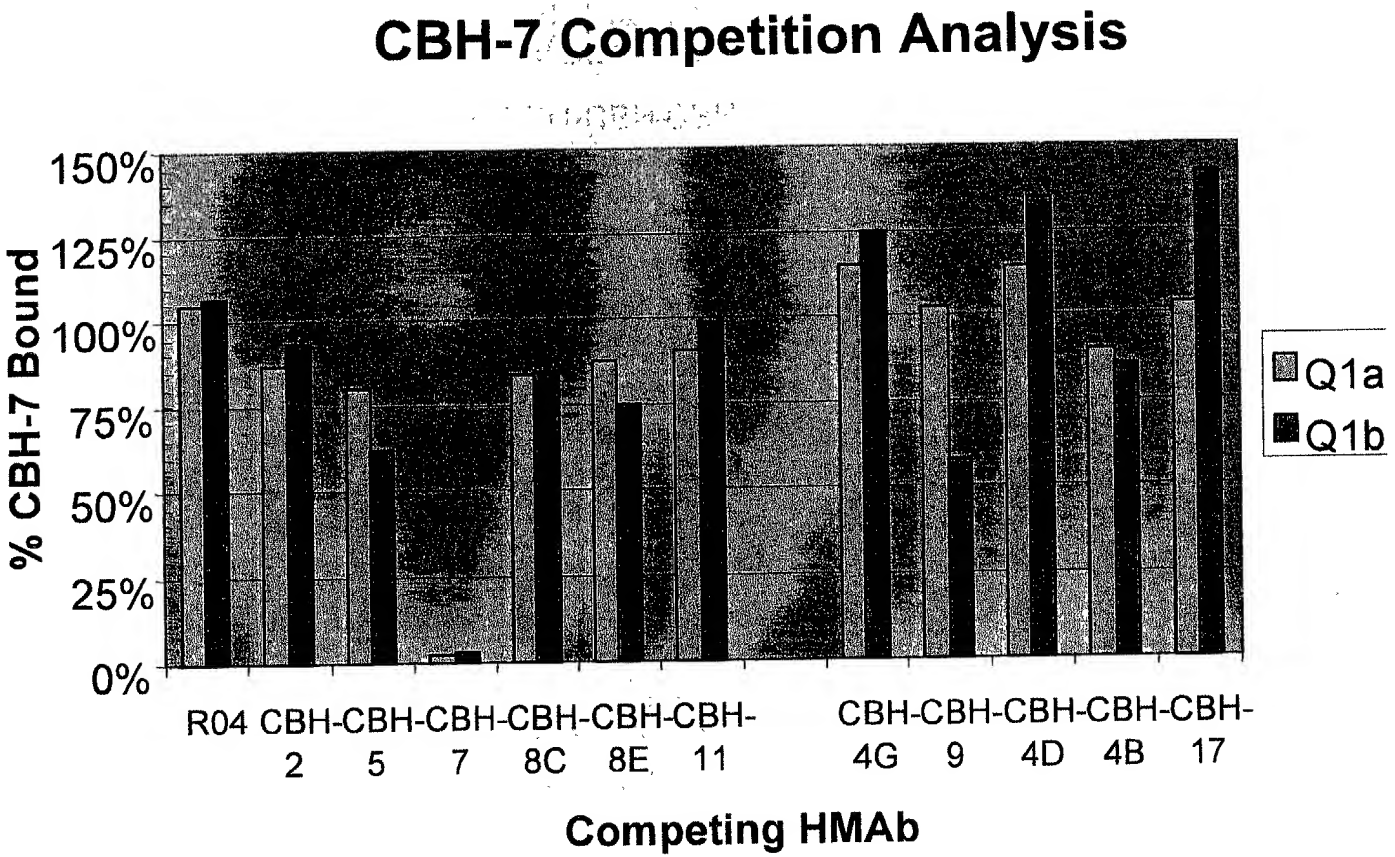
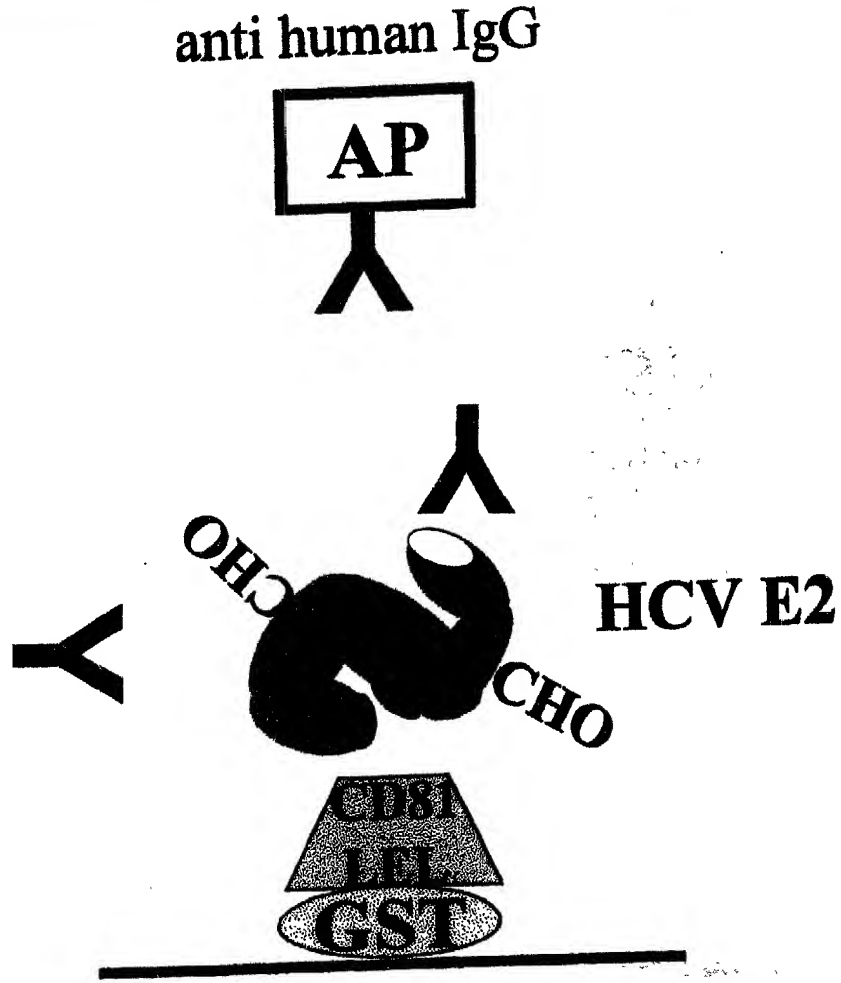

CBH-7 Competition Analysis
% CBH-7 Bound
150%
125%
100%
75%
50%
25%
0%
R04
CBH-2
CBH-5
CBH-7
CBH-8C
CBH-8E
CBH-11
CBH-4G
CBH-9
CBH-4D
CBH-4B
CBH-17
Competing HMAb
Q1a
Q1b

Figure 14

Binding to CD81-E2 complex



- Coat plates with GST-CD81-LEL or GST
- Add E2 containing protein extracts
- Add MAb
- Detect bound Ab with anti Human-AP

S.M.S.B.C
K.G.H 1999

Figure 15

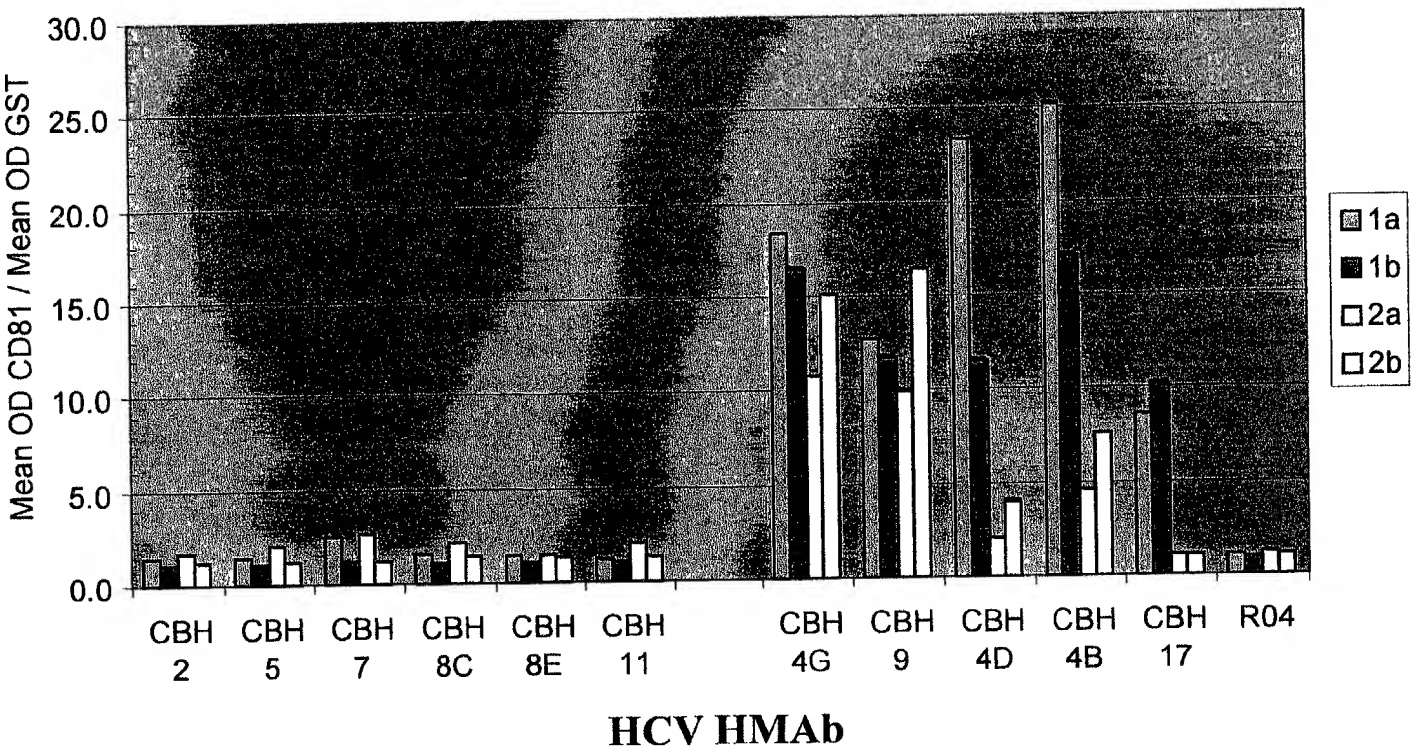
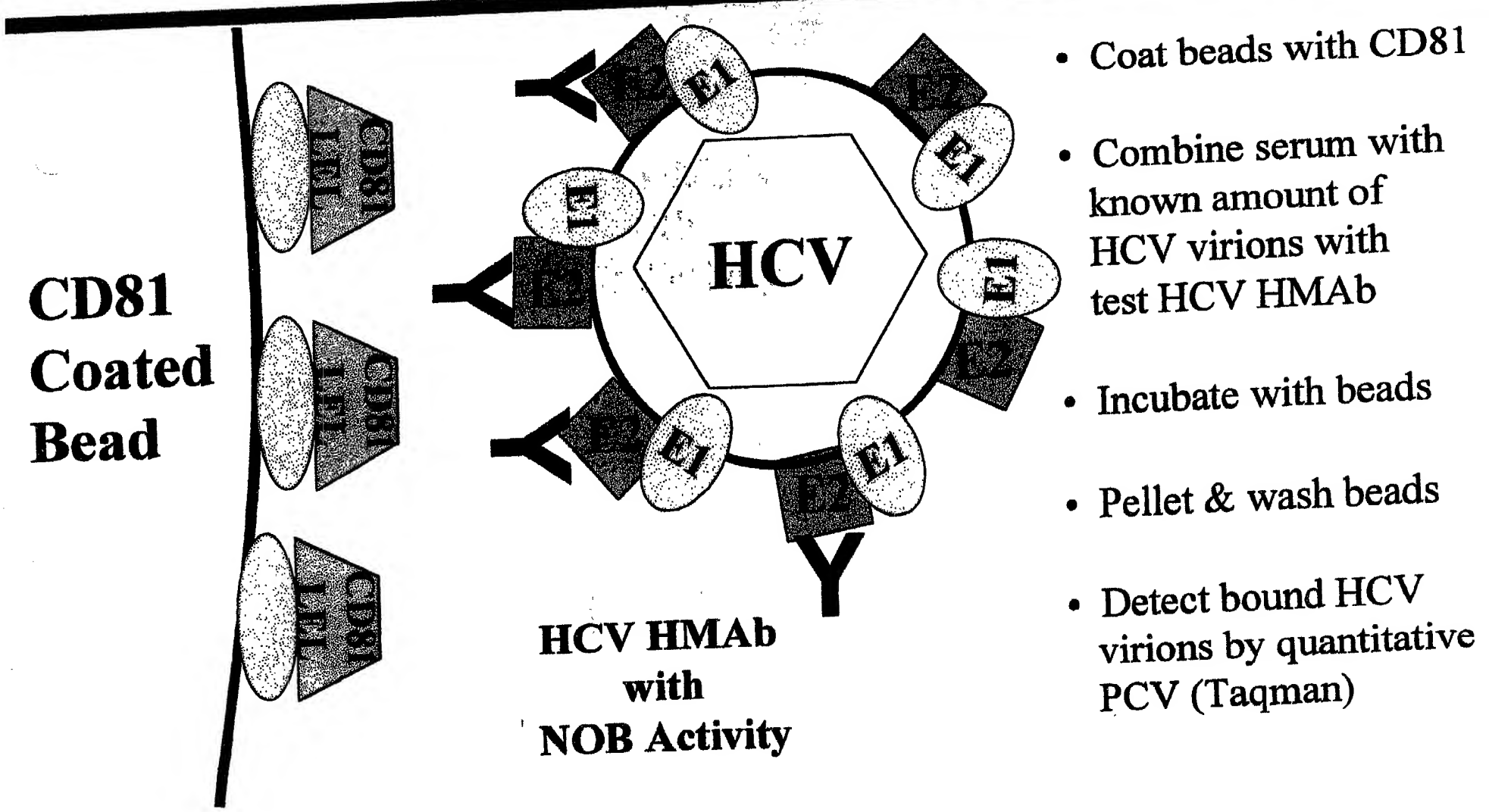


Figure 16

Inhibition of HCV virion binding to CD81



- Coat beads with CD81
- Combine serum with known amount of HCV virions with test HCV HMAb
- Incubate with beads
- Pellet & wash beads
- Detect bound HCV virions by quantitative PCV (Taqman)

Figure 17

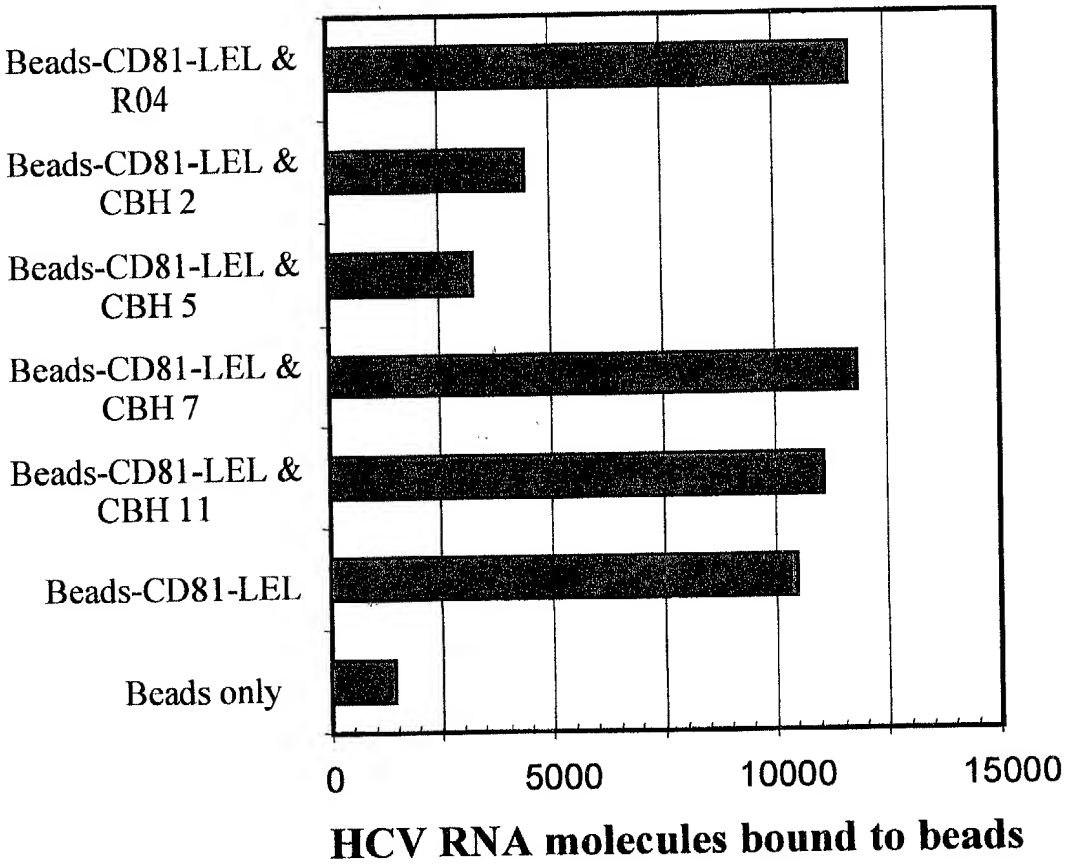

Beads-CD81-LEL & R04
Beads-CD81-LEL & CBH 2
Beads-CD81-LEL & CBH 5
Beads-CD81-LEL & CBH 7
Beads-CD81-LEL & CBH 11
Beads-CD81-LEL
Beads only
0
5000
10000
15000
HCV RNA molecules bound to beads

Figure 18

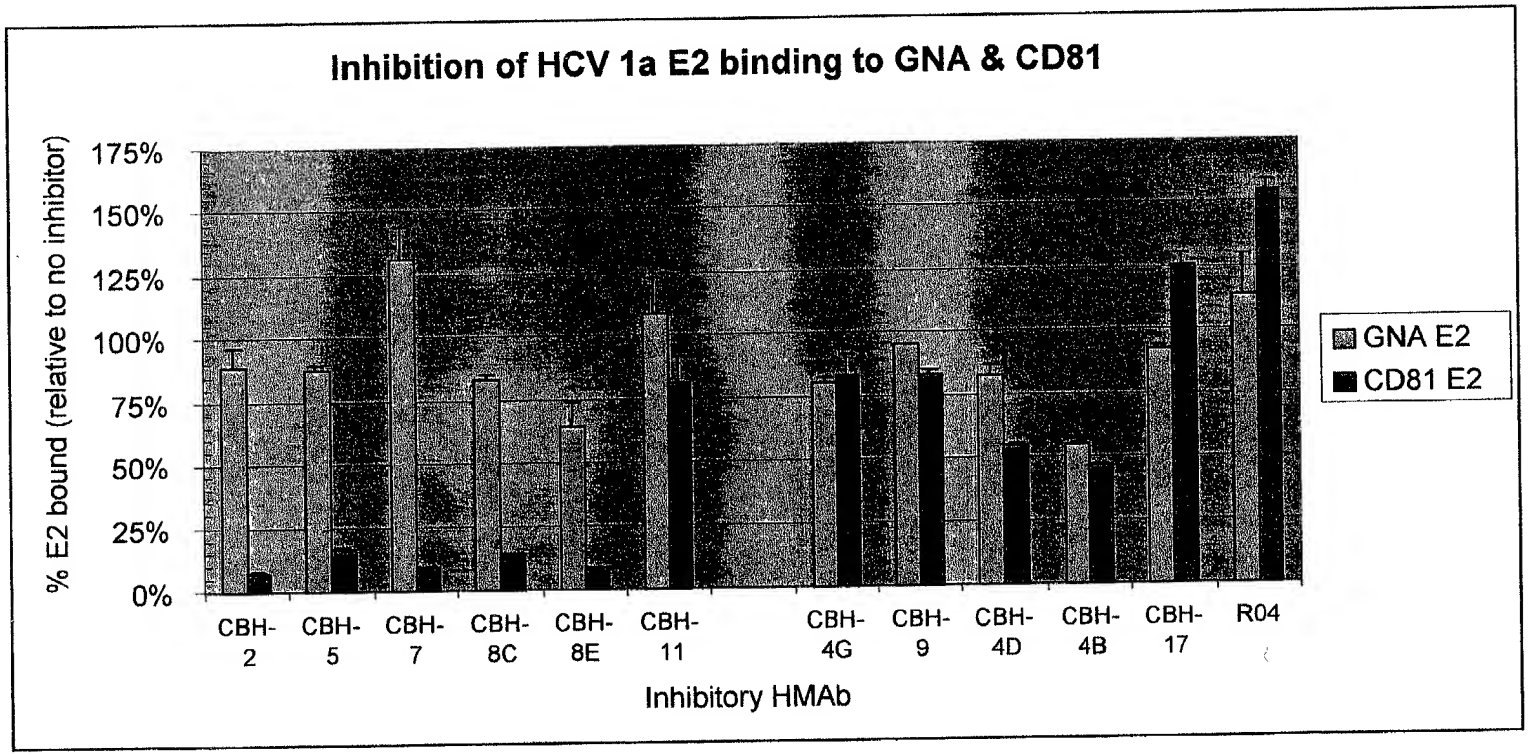


Figure 19

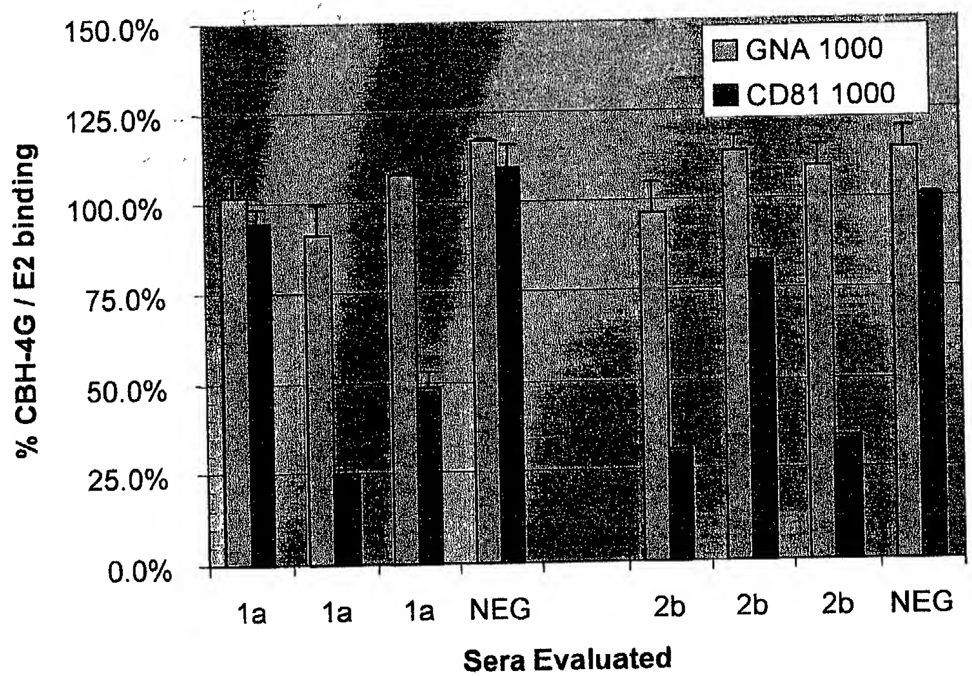
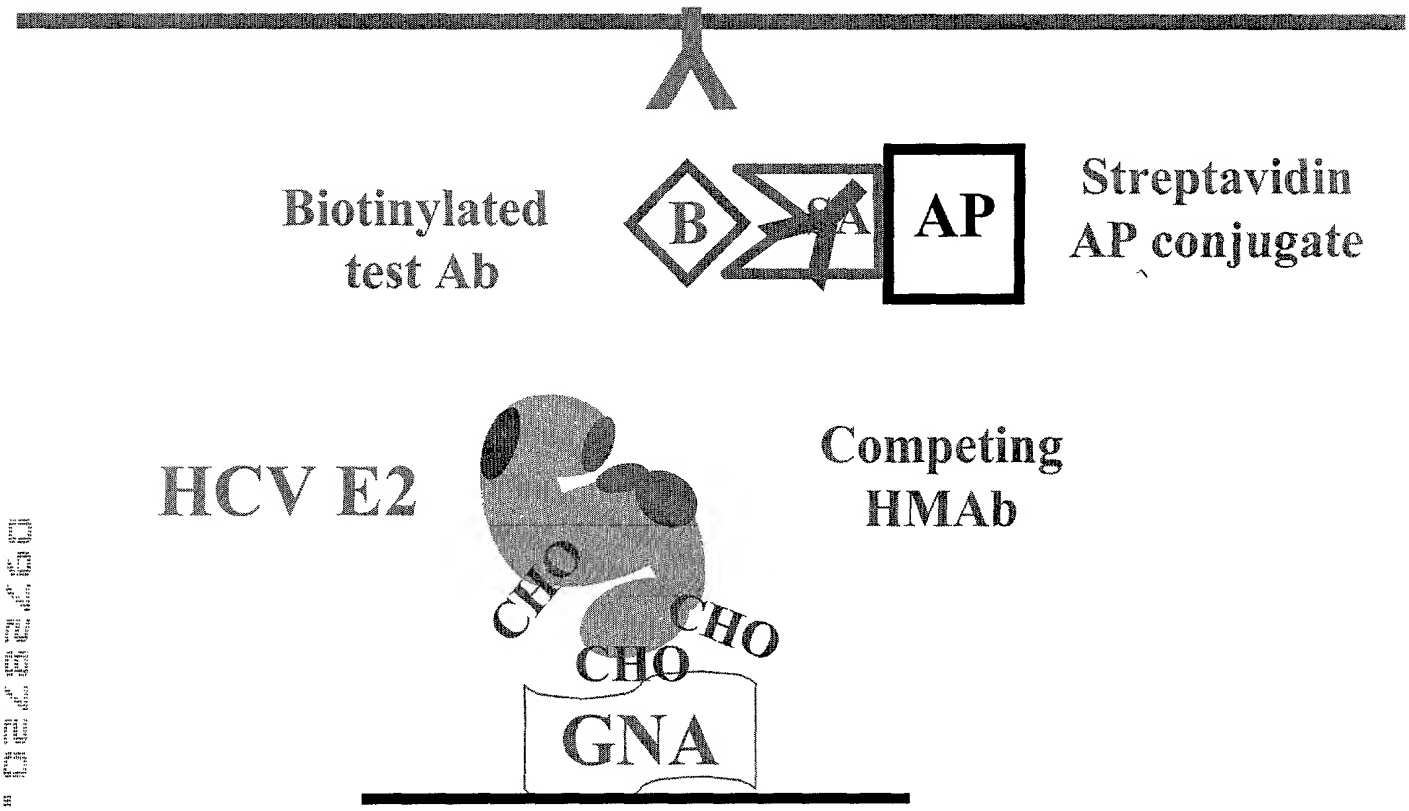

GNA 1000
CD81 1000
150.0%
125.0%
100.0%
75.0%
50.0%
25.0%
0.0%
% CBH-4G / E2 binding
1a
1a
1a
NEG
2b
2b
2b
NEG
Sera Evaluated

Figure 20

Competition Assays



- Coat plates with GNA lectin

- Capture full-length intracellular E2 onto microtiter plate by binding of CHO moieties to GNA lectin

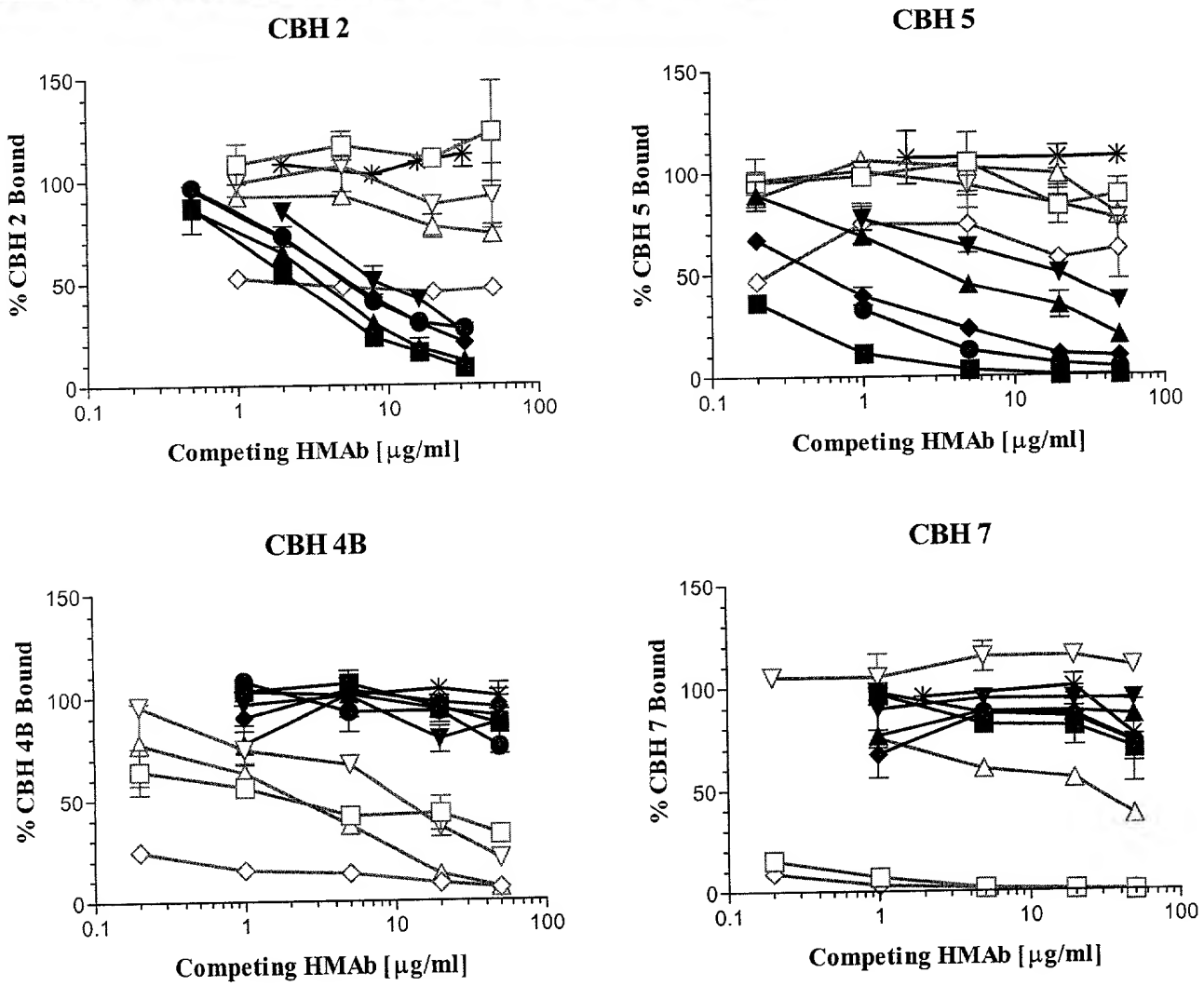
- Mix competing HMAb with GNA-captured E2

- Add biotinylated test HMAb. Detect binding of biotinylated test HMAb to E2 with streptavidin-AP conjugate

- Inhibition of binding of test HMAb suggests epitopes within same antibody binding domain

Figure 21

Competition Analysis of 4 HCV HMAbs



- CBH 2
- CBH 8E
- CBH 5
- CBH 8C
- CBH 11
- CBH 7
- XTL U68
- CBH 4G
- CBH 4B
- R04

HCV Q1b E2 protein was captured onto GNA lectin coated microtiter plates as described above. Biotinylated test antibody (indicated above each panel) at 2 μg/ml and added to wells containing the indicated concentration (x axis) of competing HMAb. Bound biotinylated test antibody detected using strepavidin alkaline phosphatase conjugate. Signal obtained in the presence of competing antibody was expressed as the percent of signal obtained by the biotinylated test antibody relative to the signal obtained in the absence of competing antibody (y axis). The points indicate the mean value obtained from 2 replicate wells. The bars indicate one standard deviation from the mean. Competing antibodies are identified in the key at left.

Figure 22

Summary of HMAb Competition Analysis

Competitor Grp	HMAb	E2	GROUP I CBH 2	CBH 5	CBH 8C	CBH 11	GRP II CBH 7	GROUP III CBH 4G	CBH 4B
I	CBH 2	1a	18	39	51	ND	93	66	76
		1b	17	50	50	48	91	84	84
	CBH 8E	1a	13	39	48	ND	79	63	80
		1b	23	45	57	51	91	87	78
	CBH 5	1a	17	9	22	ND	71	60	74
		1b	4	7	24	9	77	76	80
	CBH 8C	1a	27	48	25	ND	85	74	84
		1b	11	23	33	23	84	87	86
	CBH 11	1a	96	93	84	ND	97	72	87
		1b	24	25	43	25	82	97	83
II	CBH 7	1a	40	42	45	ND	2	251	11
		1b	104	104	89	92	2	146	36
	XTL U68	1a	60	63	108	ND	0	1	2
		1b	39	57	73	66	0	23	9
III	CBH 4G	1a	107	95	85	ND	112	40	68
		1b	87	83	81	87	114	40	44
	CBH 4B	1a	92	92	87	ND	85	24	29
		1b	78	93	66	81	63	34	13
	CBH 4D	1a	98	86	90	ND	135	37	58
		1b	91	82	76	87	102	45	37
IV	CBH 17	1a	94	87	87	ND	114	102	103
		1b	73	101	88	95	92	89	64
C	R04	1a	98	91	92	ND	101	92	98
		1b	96	104	104	101	99	120	101

Scale: > 140% | 60% - 140% | 30% - 59% | 10% - 29% | < 10%

Results are the mean percent binding of test antibody relative to wells without any competing antibody. Results are the mean values obtained from 2 – 5 separate experiments. Both genotype 1a and 1b E2 proteins were tested. ND = not done.

HCV E2 Deletion Constructs

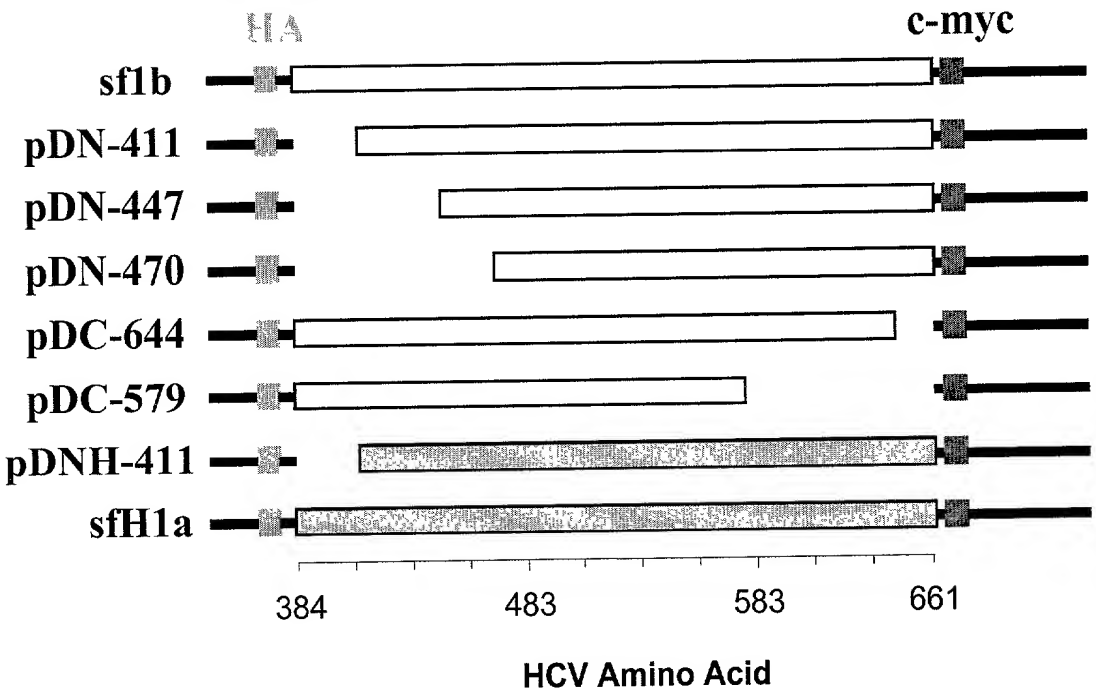
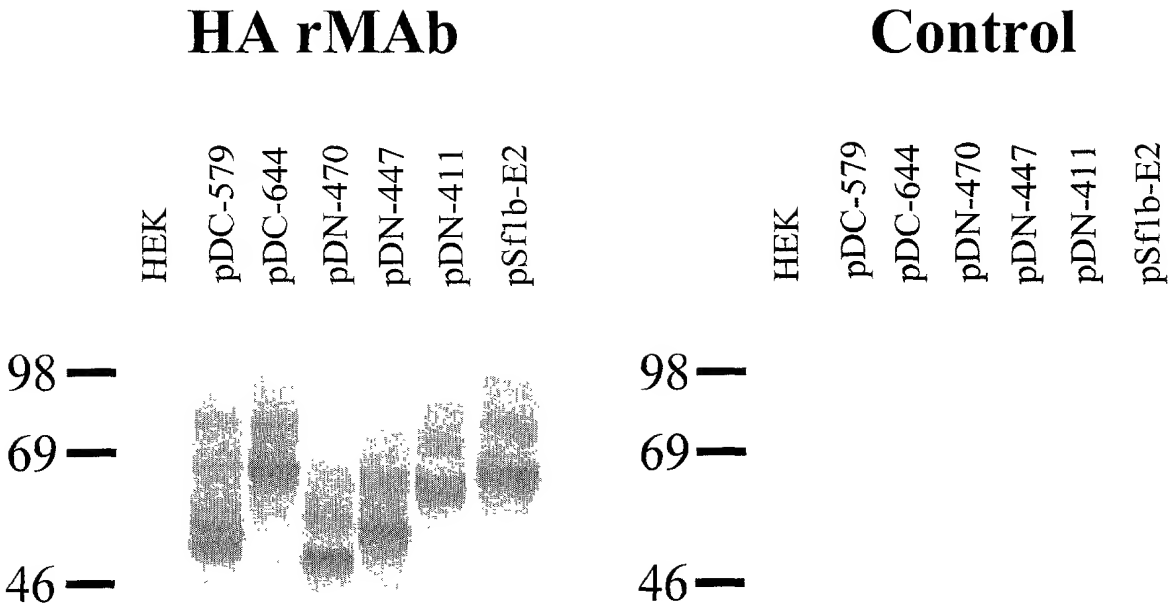


Diagram indicating sequences expressed by HCV E2 Deletion Constructs. The names of the E2 constructs are provided at left. Sequences derived from the vector pDisplay are indicated as solid black bars. The positions of the HA epitope (orange box) and the c-myc epitope (brown box) present in the pDisplay vector are also indicated. Sequences derived from HCV 1b E2 are indicated as white boxes. Sequences derived from HCV 1a strain H E2 are indicated as light gray boxes. Numbering of the X axis (below) is according to the polyprotein of the HCV-1 isolate.

Figure 23

HCV E2 Deletion Constructs are efficiently expressed

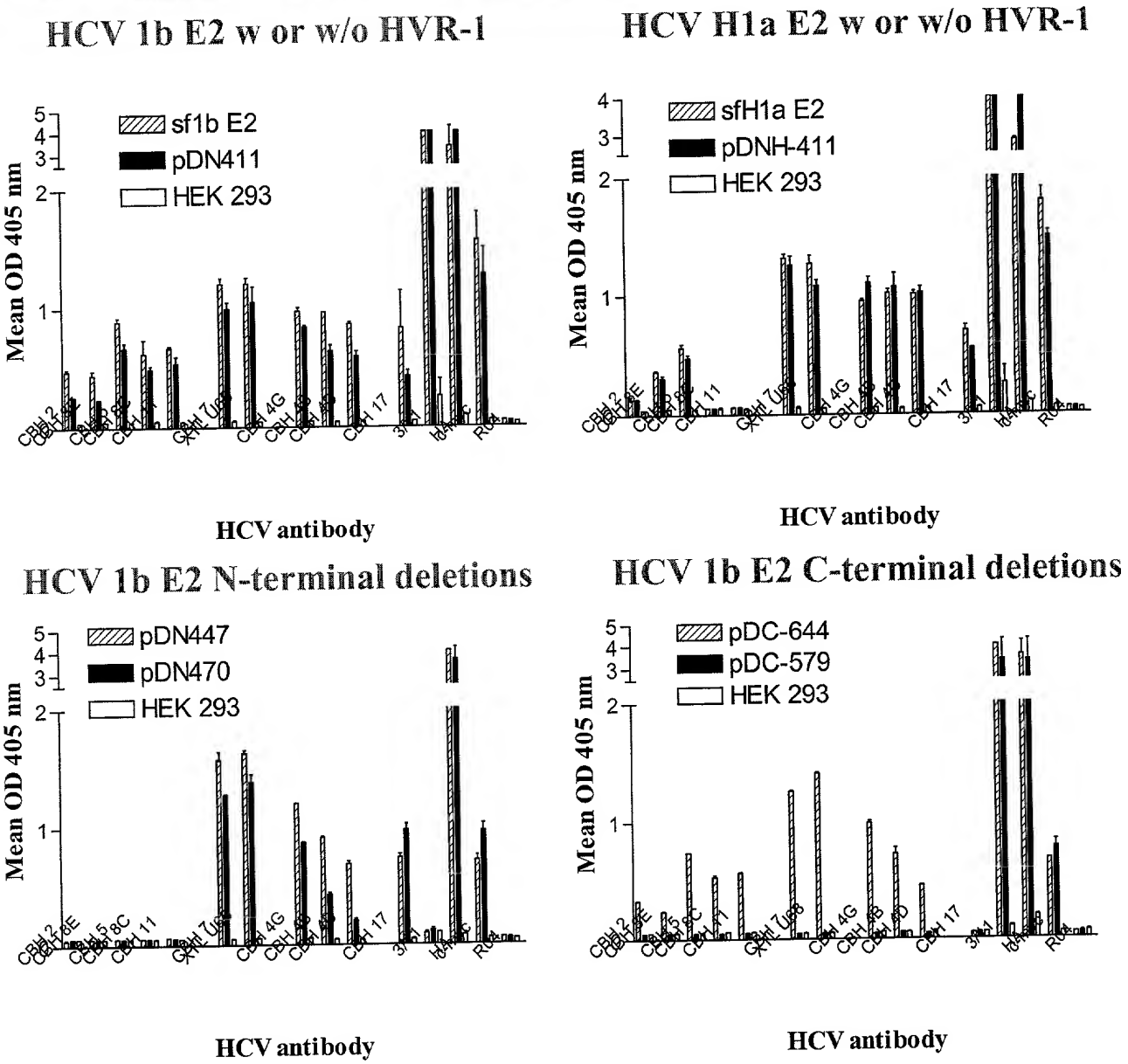


Western blot analysis of HCV E2 deletion constructs. The indicated HCV E2 constructs (above lanes) were transfected into HEK-293 cells. Twenty-four hours after transfection cytoplasmic extracts were prepared and fractionated via SDS-PAGE. The fractionated proteins were transferred to nitrocellulose membranes and incubated with either rat monoclonal antibody to the HA epitope (HA rMAb) or a control HMAb to a CMV protein (control). Bound antibody was detected with the appropriate AP conjugated antisera. HEK = mock-transfected HEK-293 cells. The migration of molecular weight markers are indicated at left.

Figure 24

Figure 25

HCV E2 Deletion Constructs



HEK-293 cells were mock transfected (white bars) or transfected with the indicated HCV E2 constructs (see keys each graph). Twenty four hours post transfection cytoplasmic extracts were prepared and equivalent aliquots were captured onto GNA lectin coated microtiter plates as described above. The captured E2 proteins were then incubated with the indicated HCV HMAb (x axis) and the amount of bound antibody determined. Bars represent the mean absorbence value obtained from duplicate wells. Error bars indicate one standard deviation from the mean.

HCV sera have variable levels of antibodies that inhibit CBH-2 & CBH-7

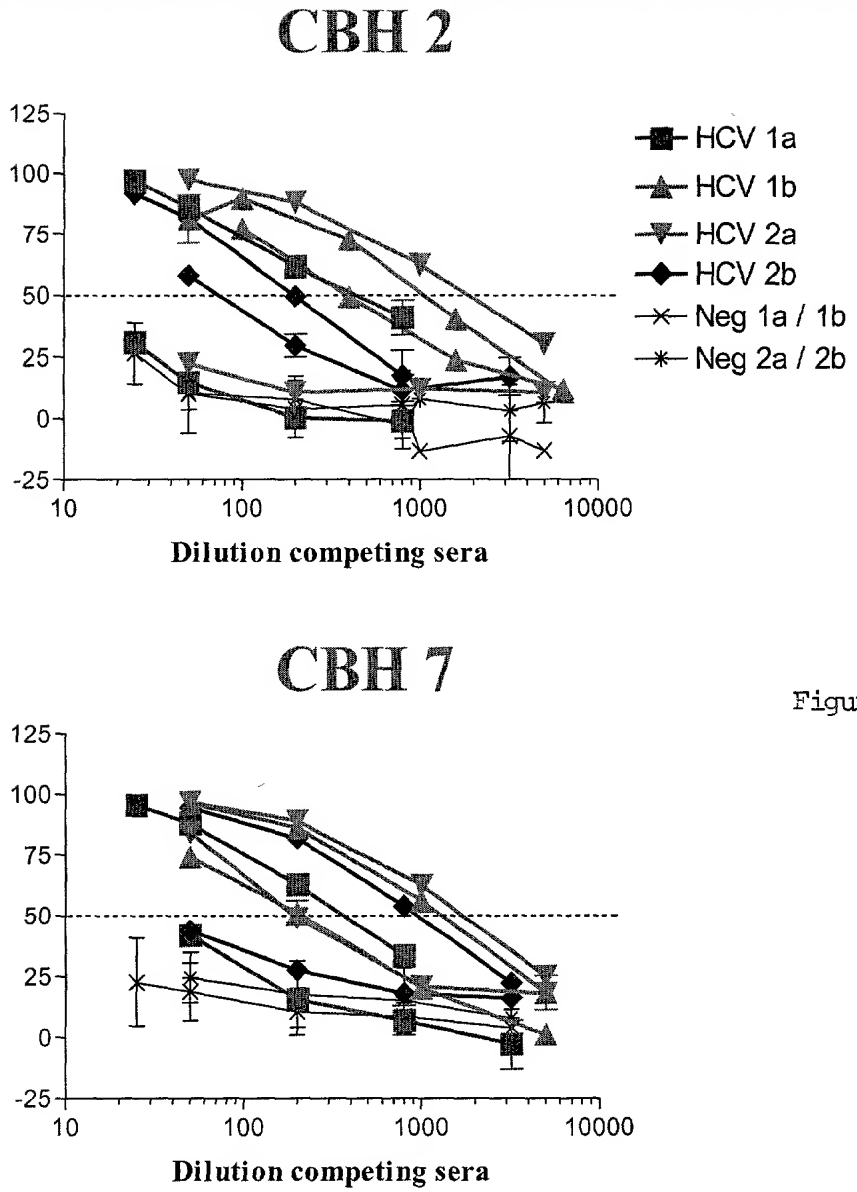


Figure 26

Homologous HCV E2 proteins were captured onto wells and incubated with the increasing dilutions of HCV 1a, 1b, 2a, or 2b sera (see key). Values are the specific inhibition of binding of biotinylated CBH-2 or CBH-7 obtained with individual sera. The mean percent inhibition (y axis) obtained from duplicate determinations at a given dilution (x axis) are plotted. The mean specific inhibition obtained for 8 negative sera are also presented (genotypes of E2 protein employed are indicated). Error bars on negative sera indicate one standard deviation from the mean.

HCV sera have variable levels of antibodies that inhibit CBH-2 & CBH-7

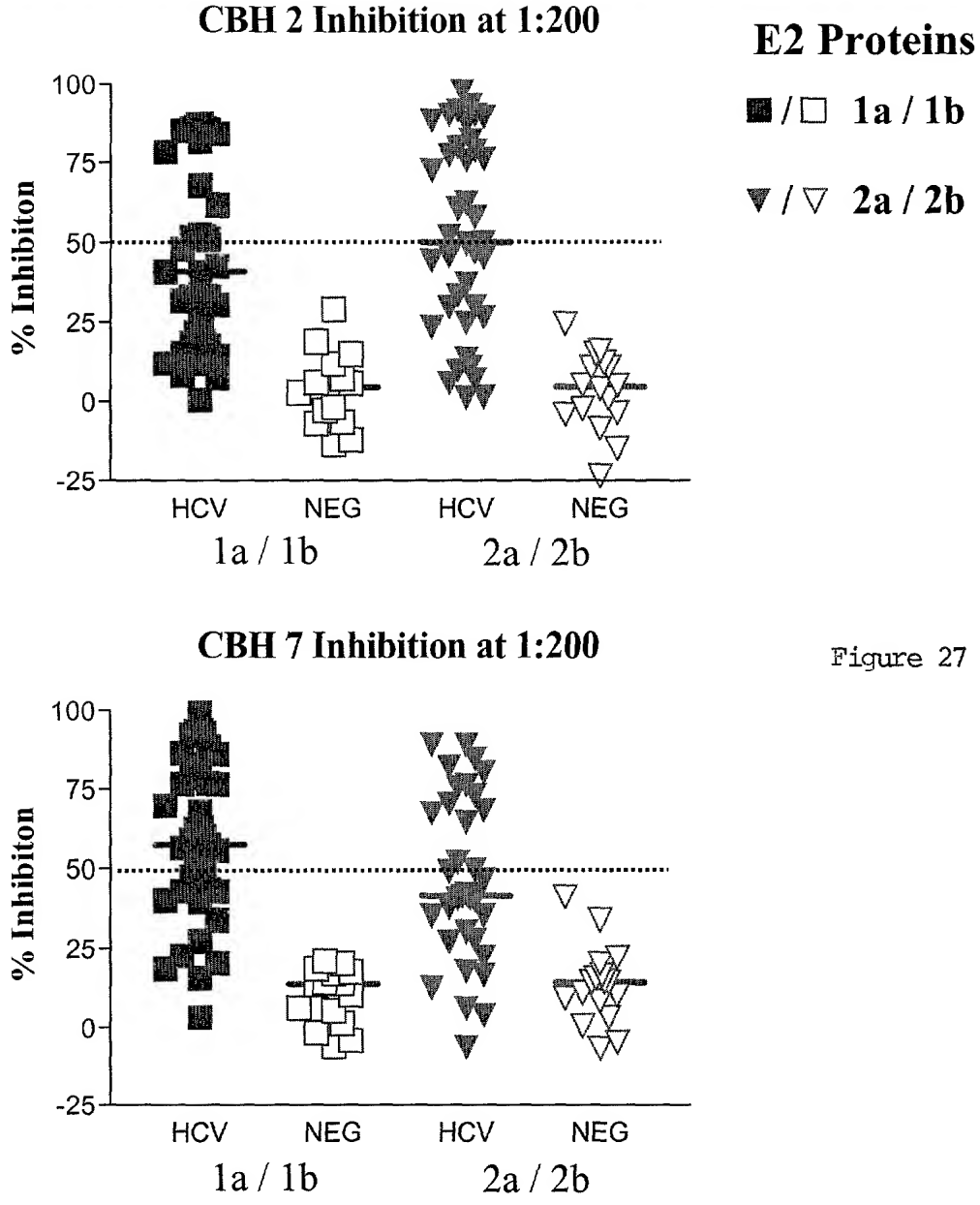
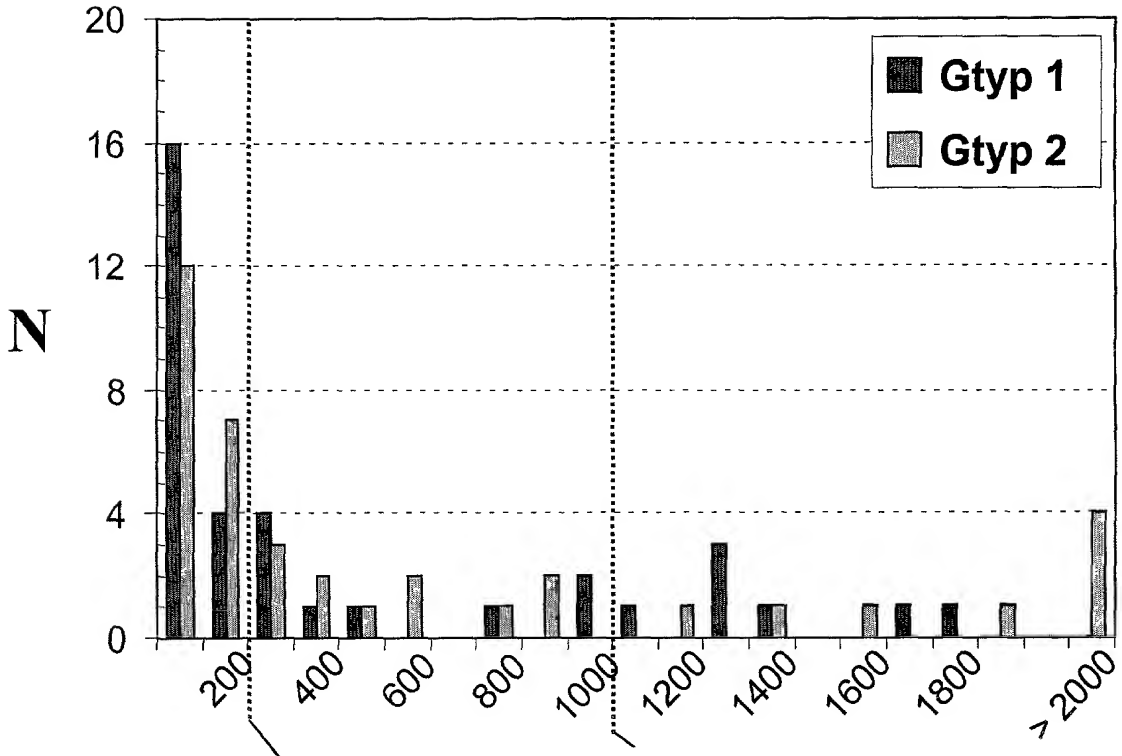


Figure 27

Scattergram showing percentage of test HMAb inhibition. HCV sera of the indicated genotype (x axis) or control sera (NEG) were diluted 1:200 and incubated with biotinylated test HMAb (indicated above graph) in wells coated with genotyped Matched E2 proteins. Binding of test HMAb was detected using strepavidin-conjugated-AP. Results obtained were compared to binding of test HMAb in absence of competitor. Each symbol indicates results obtained with an individual serum. The line indicates the median percent inhibition. The dotted line indicates the cutoff for calling a serum positive for the presence of the test HMAb.

Distribution of CBH-2 inhibitory titers in HCV Sera

Figure 28

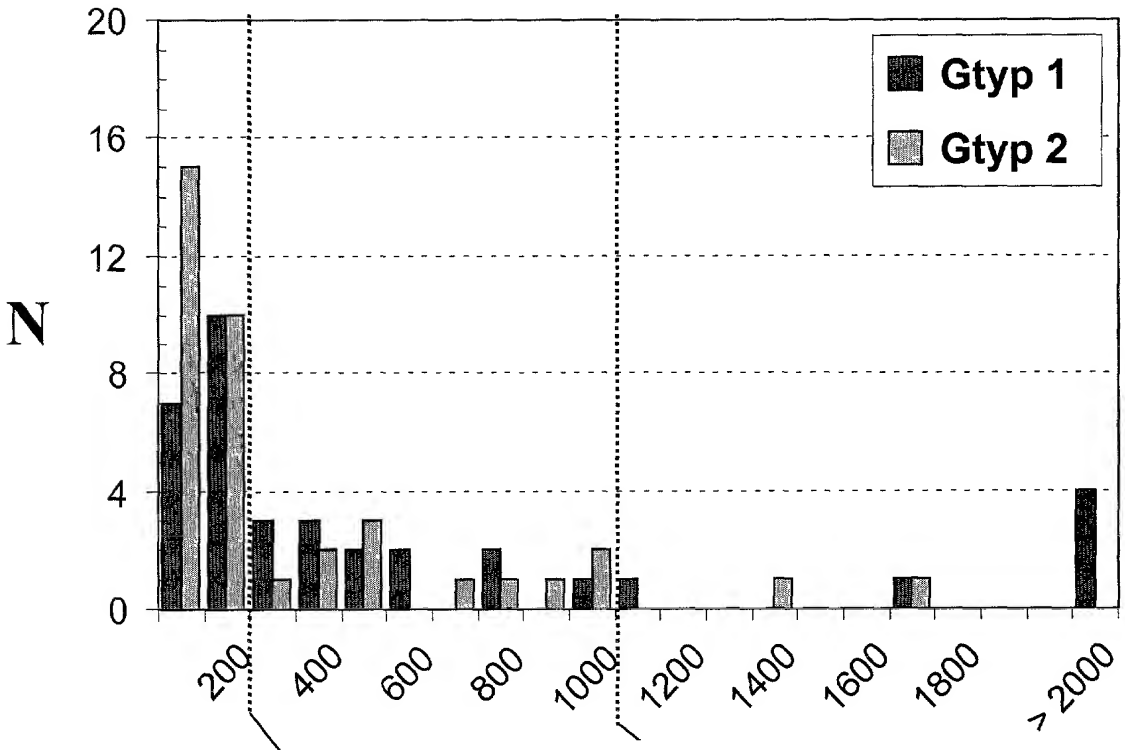


Low		Intermediate		High	
1a / 1b	20	1a / 1b	10	1a / 1b	7
2a / 2b	19	2a / 2b	10	2a / 2b	8
	53%		**27%**		**20%**

Histogram of CBH-2 inhibitory titers obtained from a panel of 74 individuals with chronic hepatitis. The CBH-2 inhibitory titers obtained with individual serum were segregated into 20 bins of 100 and 1 bind for all titers > 2000. The bars indicate the number of sera having a CBH-2 inhibitory titer within a given bin. Numbers of HCV 1a./1b sera are indicated in purple. Numbers of HCV 2a/2b sera are indicated in green. The number of sera with low (< 200) , intermediate (200-1000) and high (>1000) inhibitory titers are indicated below the graph.

Distribution of CBH-7 inhibitory titers in HCV Sera

Figure 29



Low		Intermediate		High	
1a / 1b	17	1a / 1b	13	1a / 1b	6
2a / 2b	25	2a / 2b	11	2a / 2b	2
	57%		**32%**		**11%**

Histogram of CBH-7 inhibitory titers obtained from a panel of 74 individuals with chronic hepatitis. The CBH-7 inhibitory titers obtained with individual serum were segregated into 20 bins of 100 and 1 bind for all titers > 2000. The bars indicate the number of sera having a CBH-7 inhibitory titer within a given bin. Numbers of HCV 1a./1b sera are indicated in purple. Numbers of HCV 2a/2b sera are indicated in green. The number of sera with low (< 200) , intermediate (200-1000) and high (>1000) inhibitory titers are indicated below the graph.